

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

Remarks:

Reconsideration of the application in view of the above amendments and following remarks is requested. Claims 7-9 and 12-14 are now in the case. Claims 7, 12 and 13 have been amended.

The specification has been amended to correct the title based on the election.

The specification has been amended to delete text of an embedded hyperlink.

Claims 7-9 and 12-14 stand rejected under 35 U.S.C. § 101 on the grounds that the Office does not believe that the claims are supported by a specific, substantial, and credible utility, or in the alternative, a well-established utility. The Office states that "Although it may be credible that the polynucleotide of SEQ ID NO:1 encodes a secretory protein, the lack of a specific and substantial utility, as explained above, sufficiently supports this rejection."

This ground of rejection is traversed. A *prima facie* showing of no specific and substantial credible utility must include an evaluation of *all* relevant evidence of record. The Office has further asserted that without factual evidence that the polypeptide of SEQ ID NO:2 contains a signal sequence, one skilled in the art would have reason to doubt that sequence similarity alone would reasonably support the assertion of biological activity. The Office has apparently failed to consider evidence disclosed in Applicants' specification. As taught in the specification, the polynucleotide of SEQ ID NO:1 encodes a polypeptide, Z1055G2P. Expression patterns for Z1055G2P (SEQ ID NO:1) were performed by polymerase chain reaction, for example. These results are shown in Table 6, page 17, of the specification. Specifically, the polynucleotide sequences of SEQ ID NO:1 were detected in liver, placenta, ovary, peripheral blood lymphocytes, prostate, small intestine, spleen, thymus, bone marrow, fetal liver, lymph node, and tonsil, but not in brain, heart, kidney, lung, pancreas, skeletal muscle, colon, and testis. In addition, the use of the polynucleotides of Z1055G2P as specific chromosomal probes teaches that the locus is at Yp11.2. See page 50. This locus has been shown to be the Y chromosome equivalent of the X-located cell-surface antigen defined by the monoclonal antibody 12E7. See Buckel, et al., Nature 317: 739-741, 1985. (A copy of the reference is attached.) One of ordinary skill in the art would readily comprehend that Z1055G2P polynucleotides are useful as probes for chromosomal changes linked to this region and could thus be used to detect chromosomal abnormalities. In view of the Office's apparent failure to note and evaluate

this evidence, Applicants believe that a *prima facie* showing of no specific and substantial credible utility has not been made.

The use of a nucleic acid as a probe for a specific DNA target that has been associated with a genetic disease (e.g., the Yp11.2 locus) is a credible, specific, and substantial utility. Disclosure of one specific, substantial, and credible utility meets the criteria of 35 U.S.C. § 101.

Applicants respectfully submit that the rejection is contrary to both the law and the Office's own examination guidelines. The application of these standards to biotechnology inventions is discussed in the January 5, 2001 Federal Register Notice of the Office's Utility Examination Guidelines. As stated therein:

More specifically, when a patent application claiming a nucleic acid asserts a specific, substantial, and credible utility, and bases the assertion upon homology to existing nucleic acids or proteins having an accepted utility, the asserted utility must be accepted by the examiner unless the Office has sufficient evidence or sound scientific reasoning to rebut such an assertion. "[A] 'rigorous correlation' need not be shown in order to establish utility; 'reasonable correlation' is sufficient." The Office will take into account both the nature and degree of the homology. [Citation omitted.] [66 FR 1092, 1096, Jan. 5, 2001.]

Claims 7-9, and 12-14 stand rejected under 35 U.S.C. § 112, first paragraph, on the grounds that the specification does not enable one of skill in the art to which it pertains. The Office alleges that a large amount of experimentation is necessary to determine how to use the claimed sequence. Applicants respectfully traverse this rejection. In addition to the expression and chromosomal localization results listed above, the specification teaches that the signal sequence of amino acids 1 to 21 of SEQ ID NO:2 can be used to aid in secreting fusion proteins from the cell. See, for example, page 10, lines 3-4 of the specification. All that is required is to teach one of ordinary skill in the art how to make and how to use the polynucleotides of claims 7-9 and claims 12-14. Applicants have taught how to make the polynucleotides of SEQ ID NO:1, and how to use them in expression profiles, in detecting chromosomal abnormalities, and in facilitating expression of other proteins.

Claims 7-9 and 12-14 stand rejected under 35 U.S.C. § 112, first paragraph for lack of written description on the grounds that the subject matter was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor had possession of the invention at the time it was filed. The Office alleges that while SEQ ID NO:1, its full complement, and SEQ ID NO:2 meet

the written description requirement, the open "comprising" claim language of claims 7-9 and 12-14 does not. Applicants traverse this rejection, as well. It is well-known in the field of biotechnology that a protein can be combined to additional moieties, such as spacers, engineered cleavage sites, and affinity tags. See page 13, lines 1 to 8, for example. Thus, the polynucleotides of SEQ ID NO:1 that comprise additional moieties are described in the specification.

Claims 7 and 13 stand rejected under 35 U.S.C. § 112, second paragraph, on the grounds that they are indefinite and fail to particularly point out and distinctly claim the subject matter that the Applicant regards as the invention. Claim 7 was rejected for reciting "comprising the sequence of nucleotides". Claim 7 has been amended to recite "consisting of the nucleotide sequence as shown in SEQ ID NO: 1". Claim 13 was rejected for reciting "according to" claim 12. Claim 13 has been amended to recite "of" claim 12.

Claim 7 has been rejected under 35 U.S.C. § 102 (a), on the grounds that a reference by Briggs et al., discloses a fragment of the polynucleotide as shown in SEQ ID NO:1. The cited reference discloses only a fragment of the polynucleotide sequence as shown on SEQ ID NO:1. However, Applicants believe that the afore-mentioned amendment of claim 7 obviates this rejection.

On the basis of the above amendments and remarks, Applicants believe that each rejection has been addressed and overcome. Reconsideration of the application and its allowance are requested. If for any reason the Examiner feels that a telephone conference would expedite prosecution of the application, the Examiner is invited to telephone the undersigned at (206) 442-6752.

Respectfully Submitted,

Robyn Adams
Registration No. 44,495

Enclosures:

Petition and Fee for Extension of Time (in duplicate)
Amendment Fee Transmittal (in duplicate)
1 Reference
Postcard

1. Ellison, M. L. *et al.* *Clin. Endocr.* 5, 397s-400s (1976).
2. Allan, P. M. *et al.* *Int. J. Cancer* 31, 591-593 (1983).
3. Pettengill, O. S. & Sorenson, G. D. in *Small Cell Lung Cancer* (eds F. A. Oldham, R. K. & Bunn, P. A.) 51-77 (Grune & Stratton, New York, 1983).
4. Toll, L. *J. biol. Chem.* 257, 13189-13192 (1982).
5. Stallcup, W. B. *J. Physiol., Lond.* 286, 525-540 (1979).
6. McCann, F. V., Pettengill, O. S., Cole, J. J., Russell, J. A. G. & Sorensen, G. D. *Science* 212, 1155-1157 (1981).
7. Prior, C., Lang, B., Wray, D. & Newsom-Davis, J. *Ann. Neurol.* (in the press).
8. Willcox, N., *et al.* *Hum. Immun.* 14, 29-36 (1985).

Homologous expressed genes in the human sex chromosome pairing region

V. Buckle*, C. Mondello†, S. Darling†,
J. W. Craig* & P. N. Goodfellow†

* X Chromosome Group, Genetics Laboratory,
Biochemistry Department, Oxford University, South Parks Road,
Oxford OX1 3QW, UK

† Human Molecular Genetics Laboratory, Imperial Cancer Research
Fund, PO Box 123, Lincoln's Inn Fields, London WC2A 3PX, UK

The human sex chromosomes share a pair of homologous genes which independently encode a cell-surface antigen defined by the monoclonal antibody 12E7 (refs 1, 2; see refs 3, 4 for review). The X-located homologue, *MIC2X*, escapes X-inactivation⁵ and the equivalent Y-located locus, *MIC2Y*, was one of the first genes shown to reside on a mammalian Y chromosome². By using a bacterial expression system we have previously cloned a complementary DNA sequence corresponding to a *MIC2* gene and have used this probe to show that the *MIC2X* and *MIC2Y* loci are closely related, if not identical⁶. Here we report the use of the cloned probe to confirm the localization of the *MIC2X* locus to the region Xpter-Xp22.32 (ref. 7) and demonstrate, for the first time, that the *MIC2Y* locus is located on the short arm of the Y chromosome in the distal region Ypter-Yp11.2. The *MIC2* sequences and the sequences described in the accompanying papers by Cooke *et al.*⁸ and Simmler *et al.*⁹ are the first which have been shown to be shared by the sex chromosomes in the pairing region. The Y chromosome plays a pivotal role in mammalian sex determination: its presence is associated with the male phenotype¹⁰ and its absence results in a female phenotype¹¹. This chromosomal sex determination requires that the X and Y

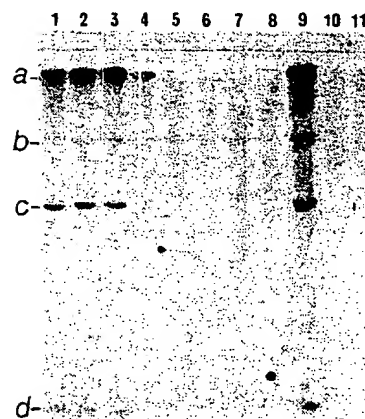


Fig. 1 Chromosomal localization of genomic sequences reacting with pSG1. Lane 1, MOLT-4 (human, XY); 2, GM1416B (human, XXXX); 3, OXEN (human YYYYY); 4, HORL9X (human-mouse hybrid, 'X only'); 5, 1W1-5 (human-mouse hybrid, 'Xq only'); 6, 445X393 (human-mouse hybrid, X-Y translocation + autosomes); 7, AMIR2N (human-mouse hybrid, X-Y translocation + autosomes); 8, UCLAB2 (human-mouse hybrid, deletion Xpter-Xp22.32 + autosomes); 9, 3E7 (human-mouse hybrid, 'Y only'); 10, RAG (mouse); 11, IR (mouse). Four bands (a-d) are visible on the original autoradiogram in all positive lanes (1-4 and 9): a, c and d, human-specific; b present in some mouse cell lines. Relative molecular mass (calculated using *Hind*III-cut $\phi\lambda$ standards, average of three determinations): a, 15.9 kb; b, 5.8 kb; c, 2.9 kb; d, 0.61 kb. Occasionally a weak band at 3.7 kb is also seen. Methods. Isolation and characterization of the pSG1 probe will be described in detail elsewhere⁶. Briefly, the cDNA sequence was isolated by screening a $\phi\lambda$ gt11 expression library with the antibody 12E7. A 1-kilobase (kb) *Eco*RI insert from a positive clone was isolated and subcloned into the vector pUC8. This clone was named pSG1. The insert from pSG1 was isolated, nick-translated to a specific activity of $\sim 2 \times 10^6$ d.p.m. μg^{-1} and used as a probe on a Southern blot. DNA preparation, cleavage with *Eco*RI, fragment separation on a 0.8% agarose gel and transfer to a nitrocellulose filter was performed using standard techniques¹². The blot was hybridized at 42°C for 16 h in the presence of 5×SSC, 40% formamide and 6% polyethylene glycol and washed to a final stringency of 0.1×SSC at 65°C. The filter was exposed to Kodak XAR5 film for 7 days at -70°C (see Table 1 for references and details of cell lines.)

Table 1 Comparison of 12E7 antigen expression and reactivity with pSG1

	Human-mouse hybrid	Human autosomes*	Human sex chromosomes*	Reaction with pSG1 (see Fig. 1)	Cell surface 12E7 antigen expression†	
					c.p.m. bound × 10 ⁻³ (s.d.)	p3X63Ag8
Experiments						
HORL9X (ref. 1)	None		X	+	24.6(0.9)	0.9(0.3)
3E7 (ref. 25)	None		Y	+	34.6(1.3)	0.6(0.1)
1W1-5 (ref. 26)	None		Xcen-Xqter	-	0.8(0.1)	0.6(0.1)
445X393 (ref. 2)	2, 3, 4, 6, 7, 11, 14, 18, 19, 21		Yqter-Yq11; Xp22.3-Xqter	-	0.4(0.1)	0.4(0.1)
AMIR2N (ref. 2)	2, 3, 4, 5, 11, 13, 14, 15, 17, 19, 20, 22		Yqter-Yq21; Xp22.3-Xqter	-	0.9(0.2)	0.9(0.2)
UCLAB2 (ref. 7)	1, 2, 3, 7, 11, 15, 17, 19, 20		Xp22.32-Xqter	-	1.5(0.1)	0.9(0.1)
Controls						
OXEN (ref. 27)	Human cell line		YYYYY	+	39.5(2.4)	1.4(0.4)
GM1416B	Human cell line		XXXX	+	29.7(2.3)	0.9(0.1)
MOLT4 (ref. 28)	Human cell line		XY	+	32.2(0.8)	0.3(0.1)
RAG (ref. 29)	Mouse cell line		-	-	0.8(0.1)	0.8(0.1)
IR (ref. 30)	Mouse cell line		-	-	1.8(0.1)	1.4(0.1)

Cell line GM1416B was purchased from the Institute for Medical Research, Camden, New Jersey.

* As determined from a combination of karyotypic and isozyme assays.

† As determined by indirect radioimmunoassay (see refs 1, 2 for details). P3.X63.AG8 is a class-matched negative-control antibody³¹; a positive reaction is arbitrarily regarded as >3 times the control value. Not all the assays were performed at the same time. s.d., Standard deviation.

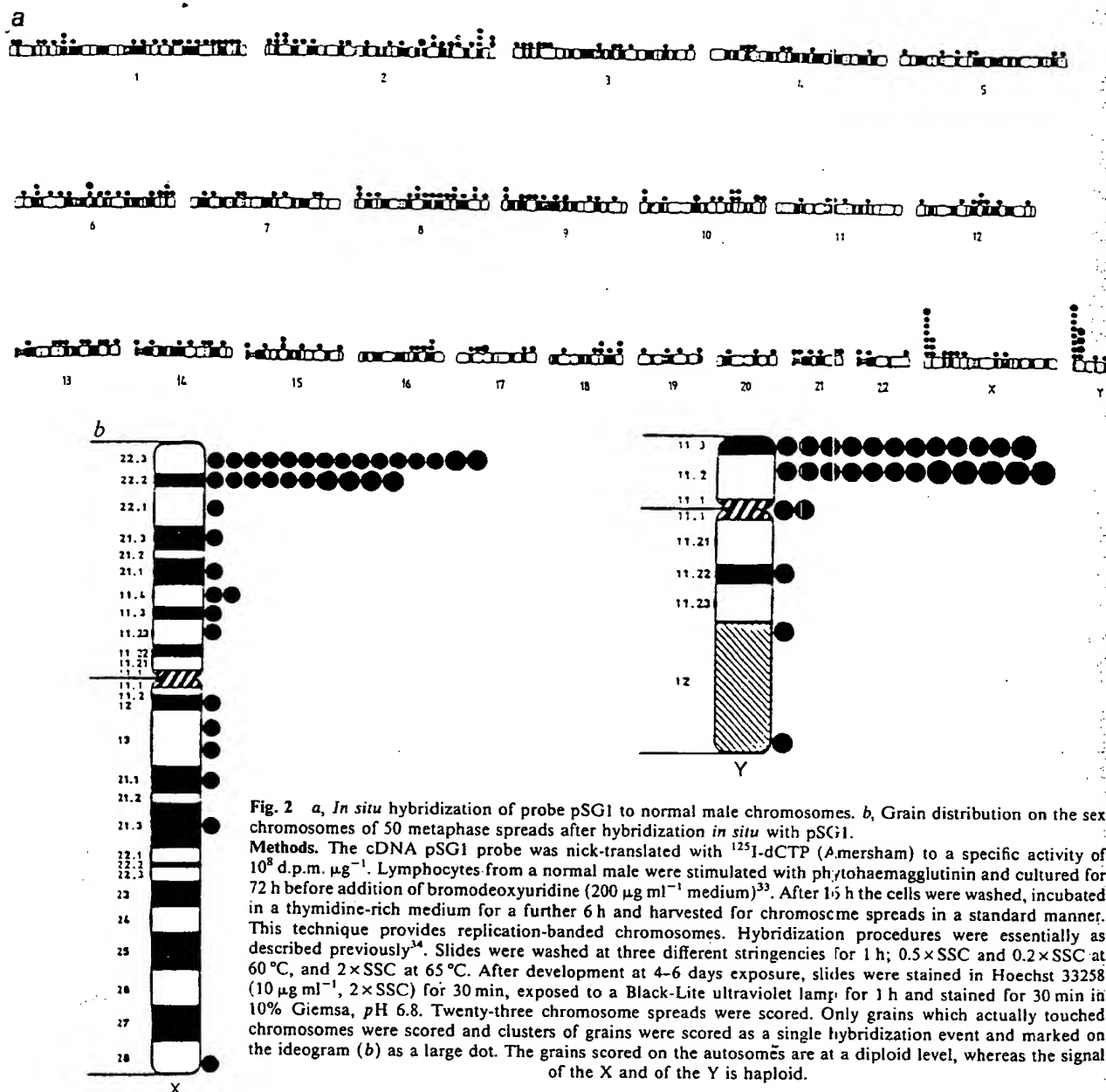


Fig. 2 *a*, *In situ* hybridization of probe pSG1 to normal male chromosomes. *b*, Grain distribution on the sex chromosomes of 50 metaphase spreads after hybridization *in situ* with pSG1. **Methods.** The cDNA pSG1 probe was nick-translated with ^{125}I -dCTP (Amersham) to a specific activity of 10^8 d.p.m. μg^{-1} . Lymphocytes from a normal male were stimulated with phytohaemagglutinin and cultured for 72 h before addition of bromodeoxyuridine ($200 \mu\text{g ml}^{-1}$ medium)³³. After 15 h the cells were washed, incubated in a thymidine-rich medium for a further 6 h and harvested for chromosome spreads in a standard manner. This technique provides replication-banded chromosomes. Hybridization procedures were essentially as described previously³⁴. Slides were washed at three different stringencies for 1 h; $0.5\times\text{SSC}$ and $0.2\times\text{SSC}$ at 60°C , and $2\times\text{SSC}$ at 65°C . After development at 4–6 days exposure, slides were stained in Hoechst 33258 ($10 \mu\text{g ml}^{-1}$, $2\times\text{SSC}$) for 30 min, exposed to a Black-Lite ultraviolet lamp for 1 h and stained for 30 min in 10% Giemsa, pH 6.8. Twenty-three chromosome spreads were scored. Only grains which actually touched chromosomes were scored and clusters of grains were scored as a single hybridization event and marked on the ideogram (*b*) as a large dot. The grains scored on the autosomes are at a diploid level, whereas the signal of the X and of the Y is haploid.

chromosomes are incorporated into different germ cells during male meiosis. The human sex chromosomes differ extensively in morphology and in genetic content¹², but meiotic pairing and segregation has been taken to indicate that the sex chromosomes still share sequences¹³. This postulated sequence homology should reside in the morphological pairing region, which occurs between the tip of the X chromosome short arm and the Y chromosome short arm^{14–16}. It has also been suggested that recombination occurs in the pairing region^{13,17} and evidence for this hypothesis has been obtained in the mouse^{18–20}. Despite the apparently compelling arguments for sequence homology in the pairing region, molecular analysis of the human X and Y chromosomes has failed to demonstrate this homology²¹, although extensive homologies were found outside the pairing region^{22–24}.

In a preliminary experiment pSG1 was hybridized to DNA samples prepared from different somatic cell hybrids which had retained either complete human sex chromosomes or defined fragments of human sex chromosomes. At high stringency on Southern blots, pSG1 hybridized strongly to human DNA and only weakly, or not at all, to mouse DNA. The probe also reacted strongly with hybrids which retained complete copies of the

human X or Y chromosome (Fig. 1, lanes 4, 9). Hybrids which retained X chromosomes with terminal short arm deletions failed to react with pSG1: the hybrid UCLAB2 (ref. 7) was particularly informative; this hybrid lacks the human-specific bands and is deleted for the terminal 2 or 3% of the X chromosome, Xpter-Xp22.32. The *MIC2Y* sequences could only be localized to the euchromatic part of the Y chromosome, Ypter-Yq11 (Fig. 1, lanes 7, 9). In these conditions of high stringency no autosomal sequences were detected which cross-reacted with pSG1 (data not shown). In all cases complete concordance was seen between cell surface 12E7 antigen expression on the hybrid and reactivity with the pSG1 probe in the Southern blots (Table 1). The results confirm the previous conclusions which relied solely on expression of the 12E7 antigen^{1,2,7}.

To localize the genomic *MIC2Y* sequence and to confirm the assignment of *MIC2X*, the pSG1 probe was hybridized *in situ* to replication-banded metaphase chromosome spreads derived from lymphocytes of a normal male. Analysis of the silver-grain distribution in 25 cells revealed two major areas of probe hybridization, Xp22.2-pter and distal Yp11.2-pter (Fig. 2*a*). 10% of all grains were scored in these regions, each comprising $\leq 0.5\%$ of the haploid human genome. Of the 22 grains scored

in these regions this was 50% scored for 1 numbers of similar. Of the bands 2 grains scored end of band. The location of the Y chromosome first form expressed has been of random sequences chromosomal *MIC2X* described *MIC2* locus. We thank B. Williams and for contribution was provided term EMI.

Received 11 Ju

1. Goodfellow
6, 777-7.

**Express
gene fi
confer:**

L. Coma
R. E. Ro
Calgene, I

The intro
advance o
allow the
crop plan
living org
remains d
herbicide
in a bacte
inhibits a
and the
Therefore
an enzym
Expressio
formed p
N-phc
broad-sp
The cellu
3-phosph
5-enolph
EPSP s
pyruvyls
shikimat
pathway
tion of :
to this c
the targ
The obje
in plant
these cc
mutant

in these regions, 5 were clusters of more than one silver grain; this was 50% of all clusters scored. Furthermore, 50 cells were scored for labelling of the sex chromosomes only (Fig. 2b). The numbers of grains scored on the distal X and Y short arms were similar. Of 38 grains scored on the X chromosome, 67% lay in the bands Xp22.2-pter. On the Y chromosome, 83% of the 29 grains scored lay between the short arm telomere and the distal end of band Yp11.2.

The localization of *MIC2* sequences to the distal short arm of the Y chromosome and the tip of the X chromosome is the first formal demonstration of sequence homology between expressed loci in the predicted pairing regions. Similar homology has been obtained by Cooke *et al.*⁸ and Simmler *et al.*⁹ studying random sequences isolated from Y chromosome cosmid libraries. Evidence has been presented that these anonymous sequences undergo meiotic exchange between the X and Y chromosomes. Based on the sequence homology between *MIC2X* and *MIC2Y*⁶ and the chromosomal localizations described here, we would predict a similar exchange of the *MIC2* loci.

We thank our colleagues J. Wolfe, G. Banting, C. Pritchard, B. Williams, G. Buttin and B. Pym for collaborating in this work and for critical reading of the manuscript. Editorial assistance was provided by C. Middlemiss. C.M. is the recipient of a long term EMBO fellowship.

Received 11 July; accepted 25 July 1985.

1. Goodfellow, P. N., Banting, G., Levy, R., Povey, S. & McMichael, A. *Somat. Cell Genet.* 6, 777-787 (1980).

2. Goodfellow, P. N. *et al.* *Nature* 302, 346-349 (1983).
3. Goodfellow, P. N. *Differentiation* 23(s), 25-39 (1983).
4. Goodfellow, P. N., Darling, S. & Wolfe, J. *J. med. Genet.* (in the press).
5. Goodfellow, P. N., Pym, B., Mohandas, T. & Shapiro, L. *J. hum. Genet.* 36, 777-782 (1984).
6. Darling, S., Banting, G., Pym, B., Wolfe, J. & Goodfellow, P. N. *Proc. nat. Acad. Sci. U.S.A.* (in the press).
7. Curry, C. J. R. *et al.* *New Engl. J. Med.* 311, 1010-1015 (1984).
8. Cooke, H. J., Brown, W. R. A. & Rappold, G. A. *Nature* 317, 687-692 (1985).
9. Simmler, M. C. *et al.* *Nature* 317, 692-697 (1985).
10. Jacobs, P. A. & Strong, J. A. *Nature* 183, 302-303 (1959).
11. Ford, C. E., Jones, K. W., Polani, P. E., de Almeida, J. C. & Briggs, J. H. *Lancet* 1, 711-713 (1959).
12. Miller, O. J., Goodfellow, P. N. & Drayna, D. *Cytogenet. Cell Genet.* 37, 176-204 (1984).
13. Burgoyne, P. S. *Hum. Genet.* 61, 85-90 (1982).
14. Pearson, P. L. & Bobrow, M. *Nature* 226, 959-961 (1970).
15. Chen, A. & Falek, A. *Nature* 231, 555-556 (1971).
16. Chandley, A. C., Goetz, P., Hargrave, T. B., Joseph, A. M. & Speed, R. M. *Cytogenet. Cell Genet.* 38, 241-247 (1984).
17. Koller, P. C. & Darlington, C. D. *J. Genet.* 29, 159-173 (1934).
18. Singh, L. & Jones, K. *Cell* 28, 215-216 (1982).
19. Evans, E. O., Burtenshaw, M. & Cattanch, B. M. *Nature* 300, 443-445 (1982).
20. Keitges, E., Rivest, M., Siniscalco, M. & Gartler, S. M. *Nature* 315, 226-227 (1985).
21. Erickson, R. P. & Goodfellow, P. N. *Nature* 311, 106-107 (1984).
22. Page, D. C., Harper, M. E., Lov, J. & Bolstein, D. *Nature* 311, 119-123 (1984).
23. Cooke, H. J., Brown, W. R. A. & Rappold, G. A. *Nature* 311, 259-261 (1984).
24. Koenig, M., Camerino, G., Heilig, R. & Mandel, J.-L. *Nucleic Acids Res.* 12, 4097-4109 (1984).
25. Marcus, M., Trantravali, R., Dev, J. G., Miller, D. A. & Miller, O. J. *Nature* 262, 65-67 (1976).
26. Hope, R. M., Goodfellow, P. N., Solomon, E. & Bodmer, W. F. *Cytogenet. Cell Genet.* 33, 204-212 (1982).
27. Bishop, C. E. *et al.* *Nature* 303, 831-832 (1983).
28. Minowada, J., Ohnuma, T. & Moore, G. E. *J. nat. Cancer Inst.* 49, 891-895 (1972).
29. Keitges, E., Rivest, M., Siniscalco, M. & Gartler, S. M. *J. Cell Biol.* 45, 74-82 (1970).
30. Nabholz, M., Miggiano, V. & Bodmer, W. F. *Nature* 223, 358-363 (1969).
31. Kohler, G. & Milstein, C. *Nature* 256, 495-497 (1975).
32. Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning* (Cold Spring Harbor Laboratory, New York, 1982).
33. Zabel, B. U., Naylor, S. L., Sakaguchi, A. Y., Bell, G. J. & Shows, T. B. *Proc. nat. Acad. Sci. U.S.A.* 80, 6932-6936 (1983).
34. Boyd, Y. *et al.* *Ann. hum. Genet.* 48, 145-152 (1984).

Expression in plants of a mutant *aroA* gene from *Salmonella typhimurium* confers tolerance to glyphosate

L. Comai, D. Facciotti, W. R. Hiatt, G. Thompson, R. E. Rose & D. M. Stalker

Calgene, 1920 Fifth Street, Davis, California 95616, USA

The introduction and expression of foreign genes in plants¹⁻³ will advance our understanding of plant molecular biology and should allow the introduction of new agronomically valuable traits into crop plants. The source of potentially useful genes is large (any living organism may be a donor), but identification of these genes remains difficult. We present here an example of how a gene for herbicide tolerance, an agronomically important trait, can be found in a bacterium and introduced into plants. The herbicide glyphosate inhibits a metabolic step in the biosynthesis of aromatic compounds and the *aroA* gene encodes the inhibited enzyme in bacteria. Therefore, we introduced a mutant allele of this gene that encodes an enzyme less sensitive to glyphosate into tobacco plants. Expression of this gene enhanced tolerance to glyphosate in transformed plants.

N-phosphonomethylglycine (glyphosate) is a widely used broad-spectrum herbicide that kills both weed and crop species. The cellular target of glyphosate in plants is probably the enzyme 5-phosphoshikimate 1-carboxyvinyltransferase (also known as 5-enolpyruvylshikimate 3-phosphate synthase, EC 2.5.1.19; EPSP synthase), which catalyses the formation of 5-enolpyruvylshikimate 3-phosphate from phosphoenolpyruvate and shikimate 3-phosphate⁴. Inhibition of this step of the shikimate pathway causes starvation of aromatic amino acids, accumulation of shikimate^{5,6} and, eventually, cellular death. Tolerance to this compound can be mediated by either overproduction of the target enzyme^{7,8} or by the presence of an altered enzyme⁹. The objective of our present work was to test whether expression in plants of a gene encoding a glyphosate-resistant EPSP synthase confers tolerance to glyphosate. The gene chosen was a mutant allele of the *aroA* locus of *Salmonella typhimurium*⁹

encoding an EPSP synthase in which amino-acid substitution of a proline to serine caused a decreased affinity for glyphosate¹⁰ without affecting the kinetic efficiency of the enzyme (G.T., unpublished observations). We used a T-DNA-based vector to express the gene in tobacco. T-DNA is a region of large plasmids, termed Ti (tumour-inducing) or Ri (root inducing) plasmids, that is transmitted to plant cells on infection by *Agrobacterium tumefaciens* and is stably integrated into the plant genome. This element carries genes that are expressed in the plant cell¹¹. The *aroA* gene of *Salmonella* has been isolated and sequenced¹⁰. To express it in plants we used two different promoters. In the first construct the *aroA* gene was fused to the transcriptional signals from the octopine synthetase gene (*ocs*)¹². A second hybrid gene, *mas-aroA*, was constructed by fusing the mannopine synthase gene (*mas*) promoter^{13,14} and the *tml*^{15,16} polyadenylation signal to the *aroA* gene. These hybrid genes, *ocs-aroA* and *mas-aroA*, are described in Fig. 1. These constructs were cloned

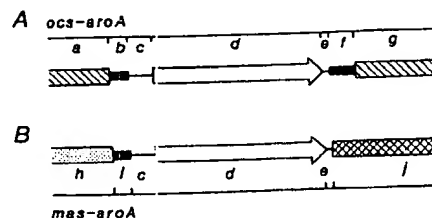


Fig. 1 Detailed structure of the *ocs-aroA* (A) and *mas-aroA* (B) genes. a, *ocs* 5', including 16 base pairs (bp) of transcribed region ending with nucleotide 13,643 (ref. 16); b, linker region, sequence GGAATCCCGG-ATCCC; c, 5'-untranslated region of *aroA*¹⁰, sequence GTTCTGTTTT-GAGAGTTGAGTTTC; d, *aroA* coding region, 1,274 bp long¹⁰; e, 3'-untranslated region of *aroA*, 17 bp, to a naturally occurring *Sal* site¹⁰; f, fragment of the tetracycline-resistance gene of pACYC184 (ref. 31), ~150 bp¹⁰ plus a portion of the pUC7 polylinker; g, *ocs* 3' starting at nucleotide 12,823 (ref. 16) and including the 3' portion of the *ocs* coding region (the *ocs* polyadenylation signal is 447 bp from the start of this region); h, *mas* 5', including ~65 bp of transcribed region ending at nucleotide 20,128 (ref. 16); i, linker region, sequence GACTCTAGAGGATCCC; j, *tml* 3' is joined to *aroA* by a *Sal*/XhoI hybrid site. An XhoI linker was spliced to the DNA termini produced by *Sma*I digestion at nucleotide 11,210 (ref. 16) of *tml* 3'; this region does not include any translated sequence and a transcription polyadenylation signal is 192 bp away. Thus, the transcribed region of the *ocs-aroA* gene is slightly longer than 1,900 bp, whereas that of the *mas-aroA* gene is slightly less than 1,600 bp long.